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Abstracts

Early Embryo Patterning

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Time-dependent patterning of the germ layers by Nodal signals

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The formation and patterning of the three germ layers are a critical early step during vertebrate embryogenesis. In all vertebrates, secreted proteins of the Nodal-related subclass of the TGF-β superfamily induce the mesendoderm and pattern all three germ layers. It is not clear, however, when these signals are required to perform each of these roles. In zebrafish, two nodal-related genes, called *squint* (*sqt*) and *cyclops* (*cyc*), have overlapping roles in mesendoderm formation. Like all Activin-like signals, Sqt and Cyc activate a bipartite receptor complex containing the ALK4 ser/thr kinase. Activated ALK4 phosphorylates the cytoplasmic factors Smad2 and Smad3, which associate with Smad4 and translocate to the nucleus where they activate transcription. To determine when activation of this pathway is required to pattern the embryo, we treated zebrafish embryos at different stages of development with SB-431542, a small molecule inhibitor of the ALK4 receptor. Our results indicate that this drug specifically blocks endogenous Nodal signals without inhibiting the ability of cells to respond to BMPs, which activate a structurally similar receptor complex. By treating embryos with SB-431542 at different developmental stages, we show that cells adopt progressively more vegetal fates when they are exposed to endogenous Nodal signals for increasing periods. Thus, our data support previous experiments suggesting that Nodal-related signals pattern the animal–vegetal, but not the dorsoventral body axis in zebrafish. In addition, our data suggest that during normal development, the effective dose of Nodal signals is regulated by how long a cell is exposed to endogenous Nodal signals.

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PTEN in zebrafish gastrulation

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PTEN is a well-characterized tumor suppressor protein with multiple functions in cell proliferation, cell polarization and cell death. PTEN is the main negative regulator of the phosphoinositol-3-kinase/protein kinase-AKT (PI3K/AKT) pathway, which plays a key role in zebrafish early development. To test whether or not Pten regulates cell movements during gastrulation, we first cloned two paralogous pten genes from zebrafish. Sequence analysis showed that they are identical to the Ptena and Ptenb isoforms reported by Croushore et al., 2005. RT-PCR analysis showed that ptena and ptenb are maternally derived and ubiquitously expressed during early stages, and that each gene is alternatively spliced. Overexpression results showed that Ptena produced a higher frequency of phenotypic effects than Ptenb. Since a similar trend was seen when the RNAs were expressed off the HSP promoter, subsequent studies focused on the longest variant, Ptena. Ptena, when expressed in U87MG human glioma cells, decreased the level of phosphorylated AKT. In embryos, ptena overexpression caused similar effects to those induced by the PI3K inhibitor wortmannin. Ptena-induced phenotypes included cardia bifida, heart edemas, defective otoliths, small eyes and/or the loss of anterior or posterior structures. Further analysis using in situ hybridization and probes to hgg, ntl and krox 20 showed altered expression patterns by 9 hpf and suggest that a delay in gastrulation and abnormal convergence is the likely cause of the perturbations. Together, these results implicate the PI3K/AKT pathway plays a key role during gastrulation, participating in the coordinated cell movements that influence subsequent development.

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Uncovering genes essential for neuronal development in zebrafish using a GFP-based forward genetic screen

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Neuronal development in vertebrates is a complex multistep process beginning with irreversible commitment of naïve ectodermal cells to a neural fate followed by proliferative precursors exiting the cell-cycle and undergoing terminal differentiation. Expression of the neural-specific α1tubulin gene spans the entire developmental phase of zebrafish neurons such that a GFP reporter driven by its

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